

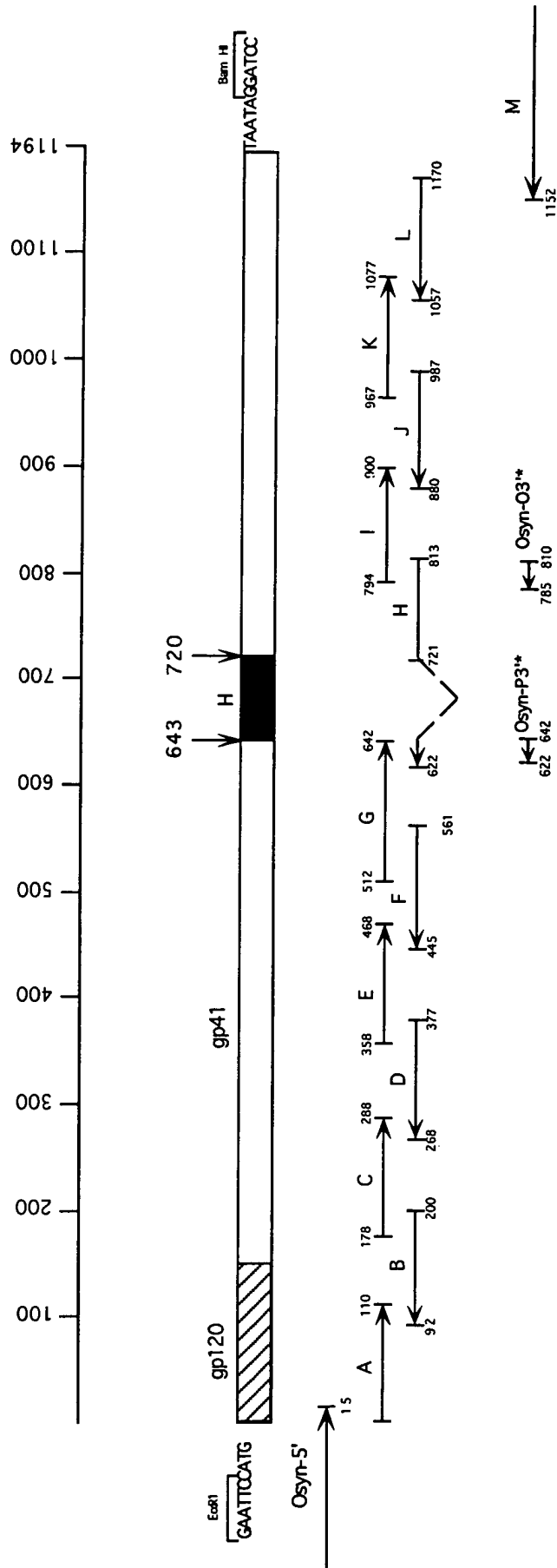
# Figure 1

→ gp120  
MIVTMRAMGK RNRKLGILYI VMALIIPCLS SSQLYATVYA GVPVWEDAAP 50  
VLFCASDANL TSTEKHNVA SQACVPTDPT PHEYLLTNVT DNFNIWENYM 100  
VEQMQEDIIS LWDQSLKPCI QMTFMCIQMN CTDIKNNNTS GTENRTSSSE 150  
NPMKTCEFNI TTVLKDKKEK KQALFYVSDL TKLADNNTTN TMYTLINCNS 200  
TTIKQACPKV SFEPPIPIYYC APAGYAIFKC NSAEFNGTGK CSNISVVTCT 250  
HGIKPTVSTQ LILNGTSLKE KIRIMGKNIS DSGKNIIVTL SSDIEITCVR 300  
PGNNQTVQEM KIGPMAWYSM ALGTGSNRSR VAYCQYNTTE WEKALKNTAE 350  
RYLELINNTE GNTTMIFNRS QDGSDVEVTH LHFNCHGEFF YCNTSEMFNY 400  
TFLCNGTNCN NTQSINSANG MIPCKLKQVV RSWMRGGGSL YAPPIPGNLT 450  
CISHITGMIL QMDAPWNKTE NTFRPIGGDM KDIWRNELFK YKVVRVKPFS 500  
VAPTPIARPV IGTGTHREKR → gp41  
AVGLGMLFLG VLSAAGSTMG AAATALTVQT 550  
HSVIKGIVQQ QDNLLRAIQA QQELLRLSVW GIRQLRARLL ALETLIQNQQ 600  
LLNLWGCKGR LICYTSVKWN ETWRNTTNIN QIWGNLTWQE WDQQIDNVSS 650  
TIYEEIQKAQ VQQEQNEKKL LELDEWASLW NWLDITKWLW YIKIAIIIIVG 700  
ALIGVRIVMI VLNLVRNIRQ GYQPLSLQIP TRQQSEAETP GRTGEGGGDE 750  
GRPRLIPSPQ GFLPLLYTDL RTIILWSYHL LSNLISGTQT VISHLRLGLW 800  
ILGQKIIDAC RICA AVIHYW LQELQKSATS LIDTFAVAVA NWTDDIILGI 850  
QRLGRGILNI PRRVRQGFER SLL 873

0011004-031597

Figure 2

# Diagram of Oligonucleotides Used to Generate Synthetic Gene Constructs



\* Translational terminators and Bam HI cloning sites on the 5' end

pGO-8 insert = Osyn-5' to Osyn-P3'  
 pGO-9 insert = Osyn-5' to Osyn-O3'  
 pGO-11 insert = Osyn-5' to Osyn-M  
 H = Hydrophobic region (deleted as shown)

5' → 3'  
 3' ← 5'

# Construction of pGO-9PL/DH5 $\alpha$ and pGO-9CKS/XL1

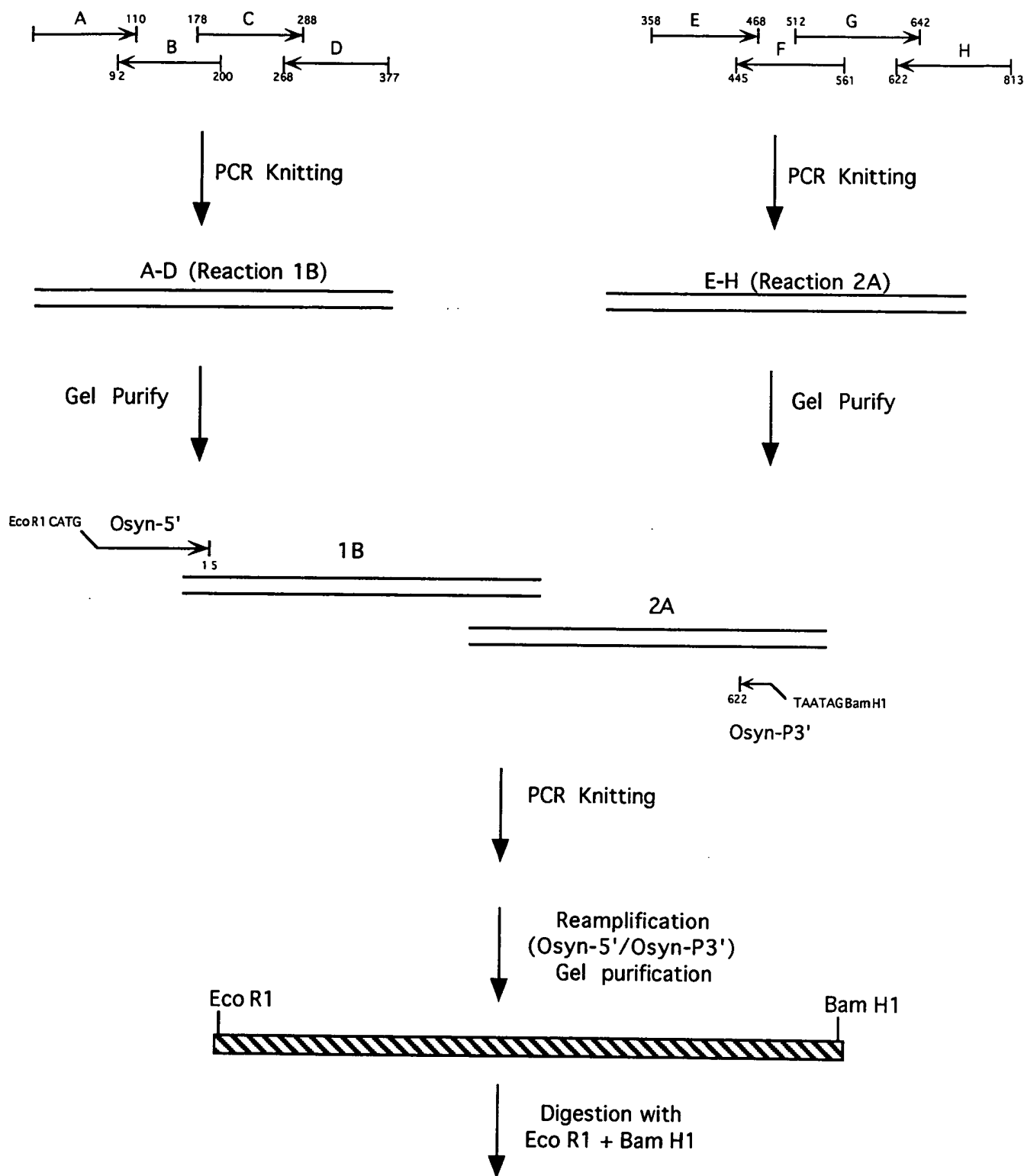
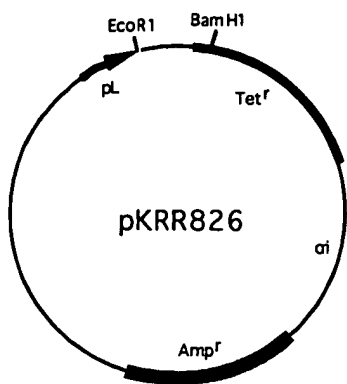


Fig 3A

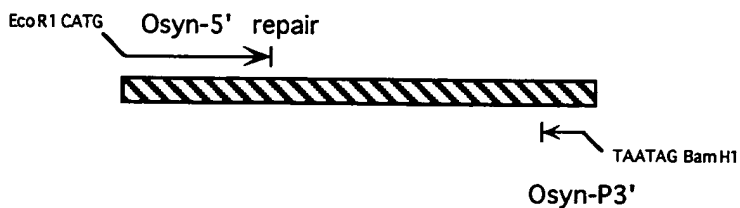


Eco R1 + Bam H1 digestion

Gel Purify

Ligate insert +  
Vector

Transformation  
of DH5 $\alpha$



PCR Modification  
(Osyn-5' repair +  
Osyn-P3') of A2

Gel isolation

Digestion (Eco R1 +  
Bam H1)



pKRR 826  
Eco R1 + Bam H1  
Digested

Ligate insert +  
Vector

Transformation  
of DH5 $\alpha$

Fig 3B

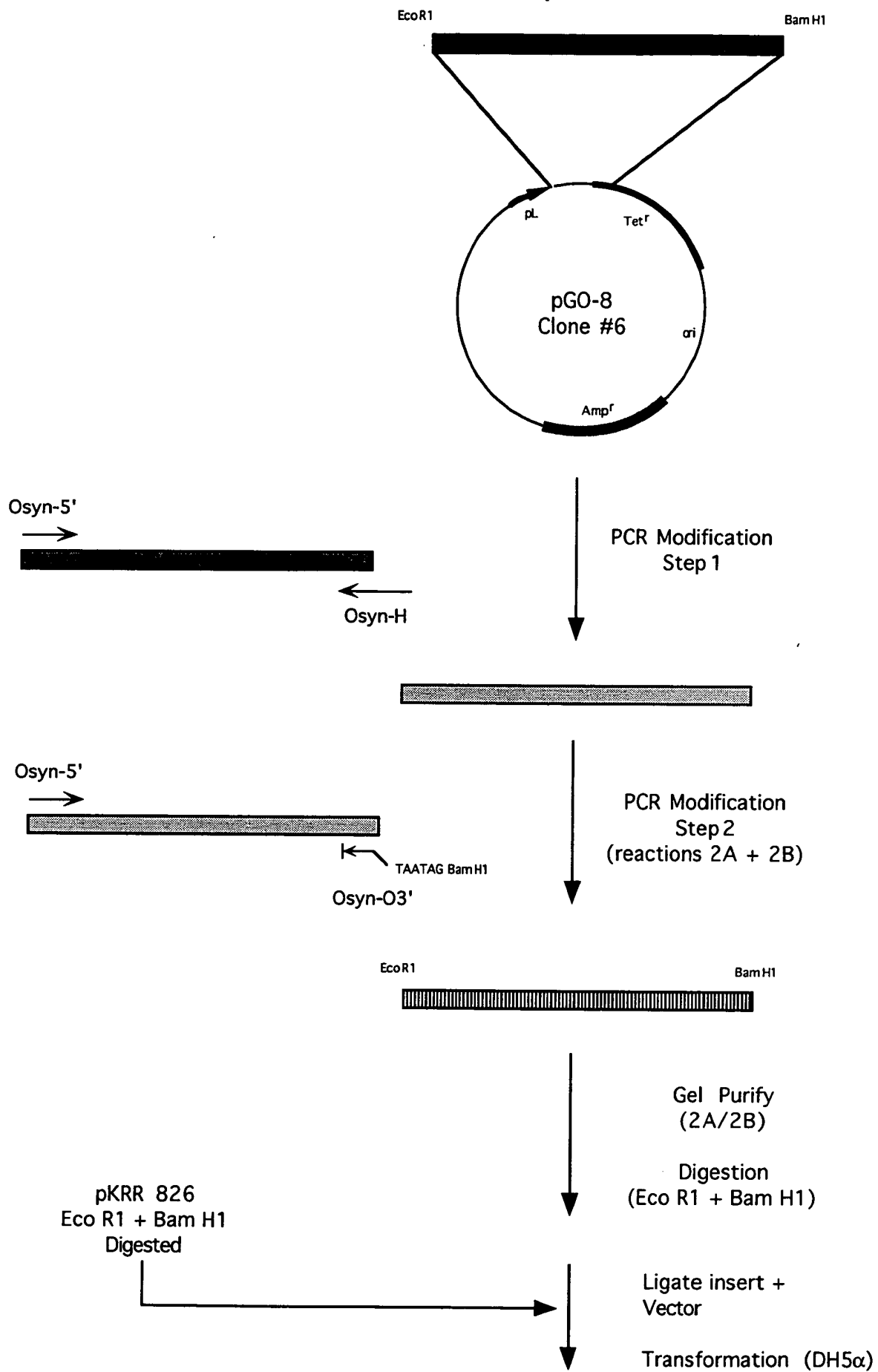


Fig 3C

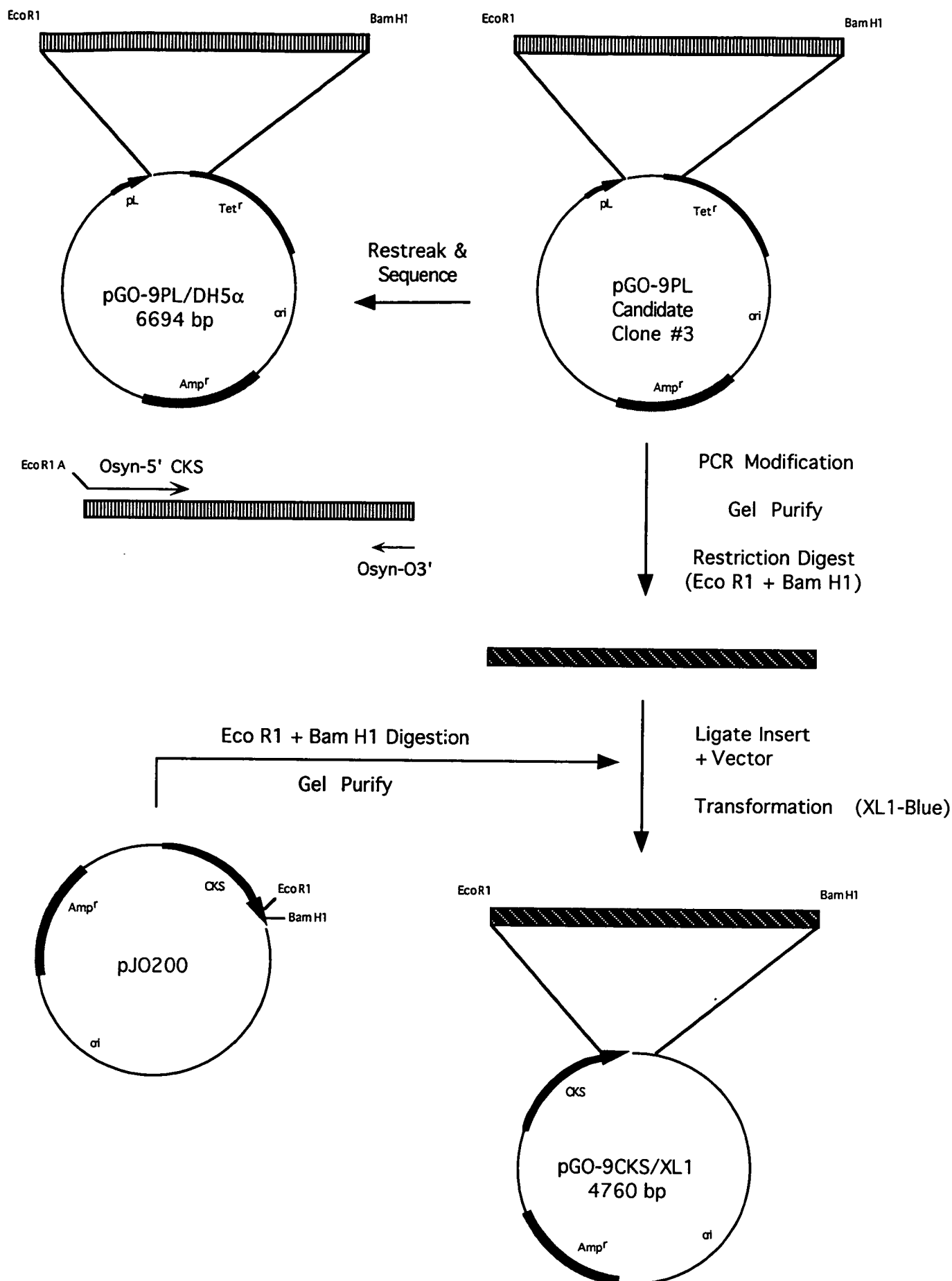


Fig 3D

# Construction of pGO-11PL and pGO-11CKS Synthetic Genes

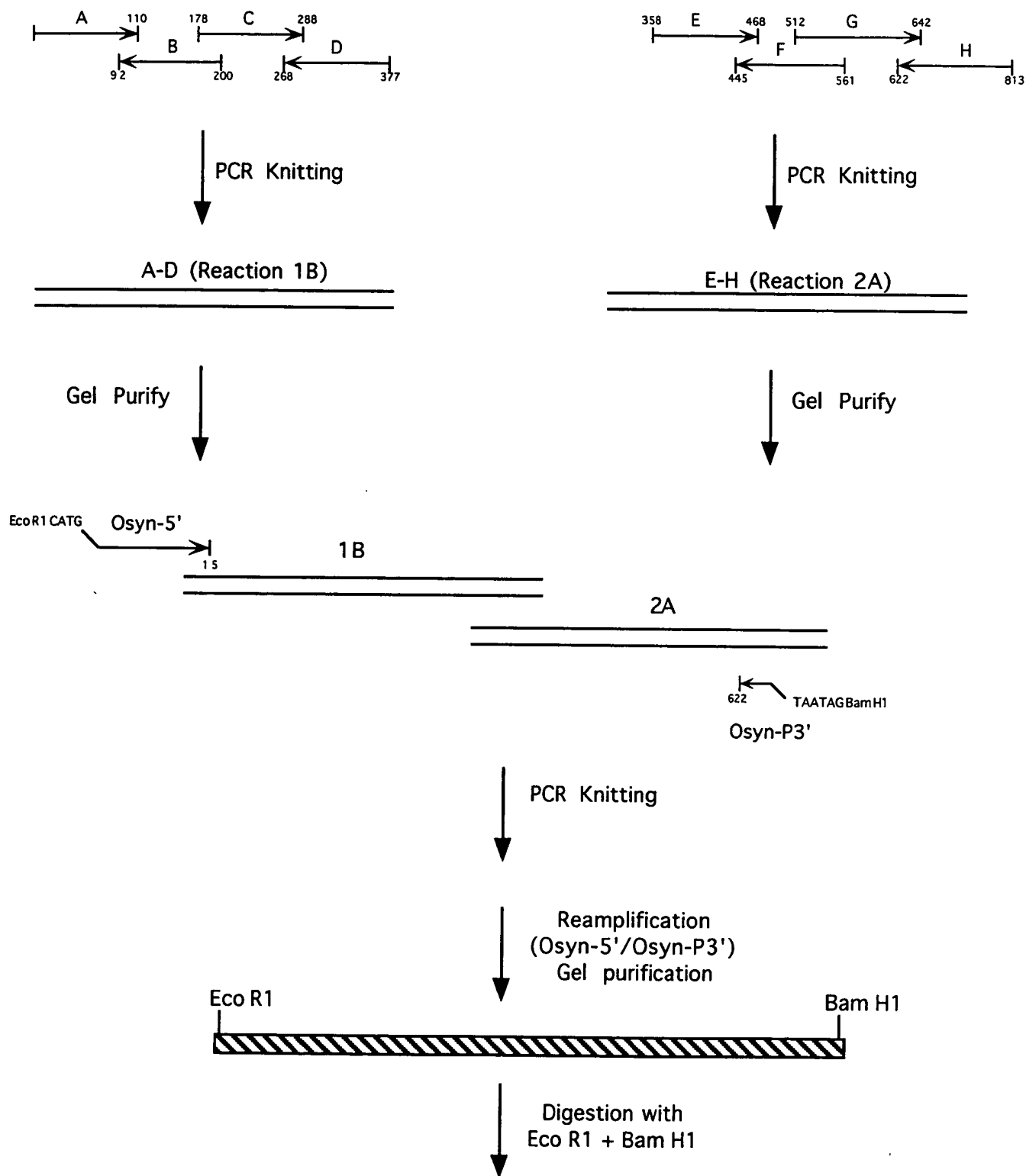


Fig 4A

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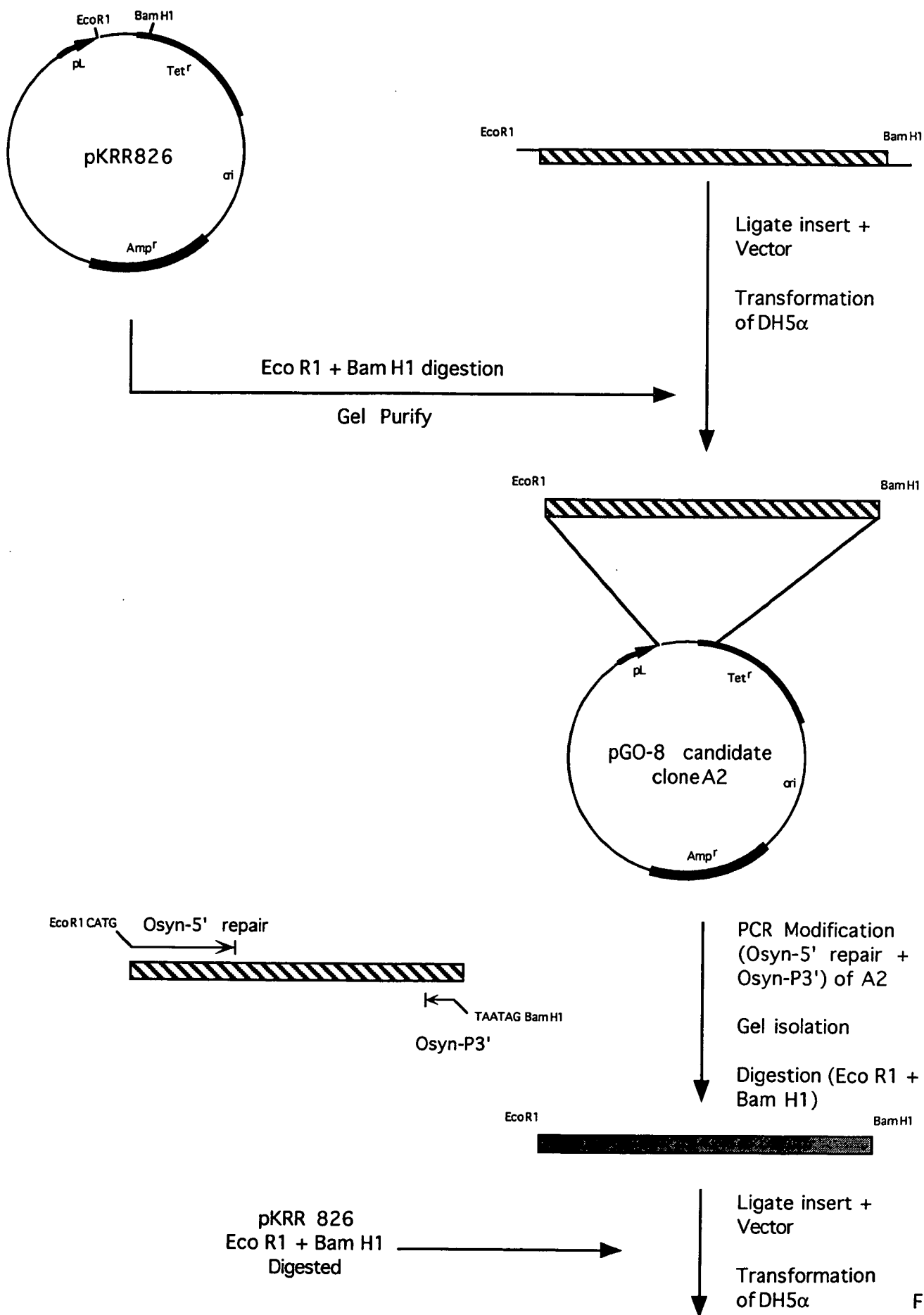


Fig 4B



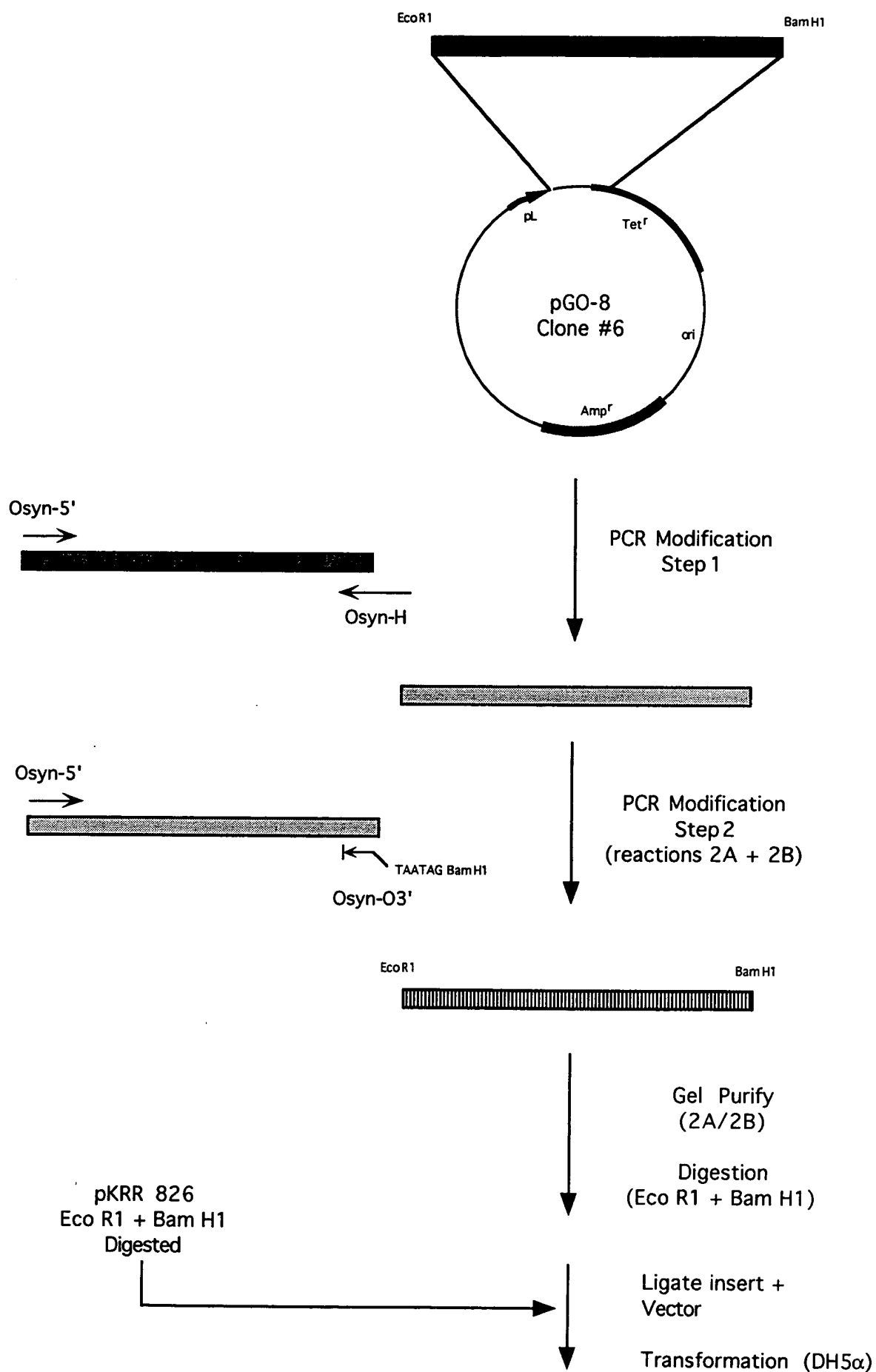
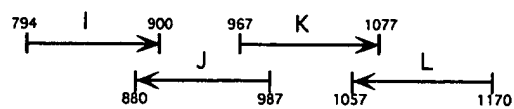
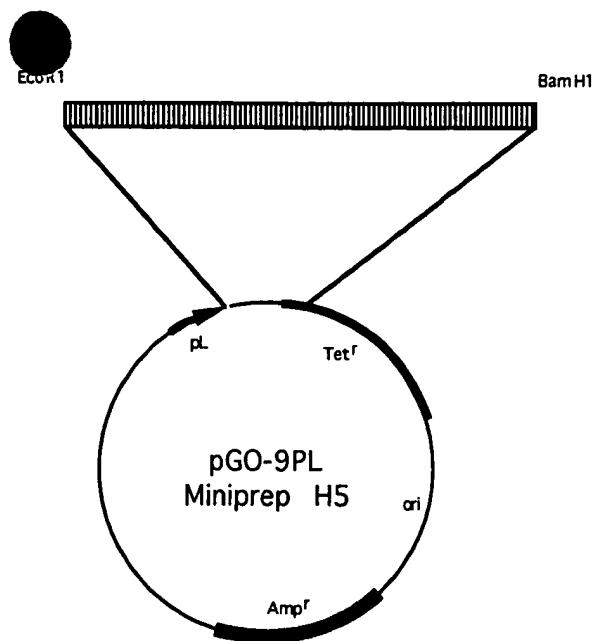


Fig 4C

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PCR Knitting

I-L (Reaction 3A)

Gel Purify

I-PCR

3A

M

1152

PCR

Gel Purify

Osyn I-M



Fig 4D

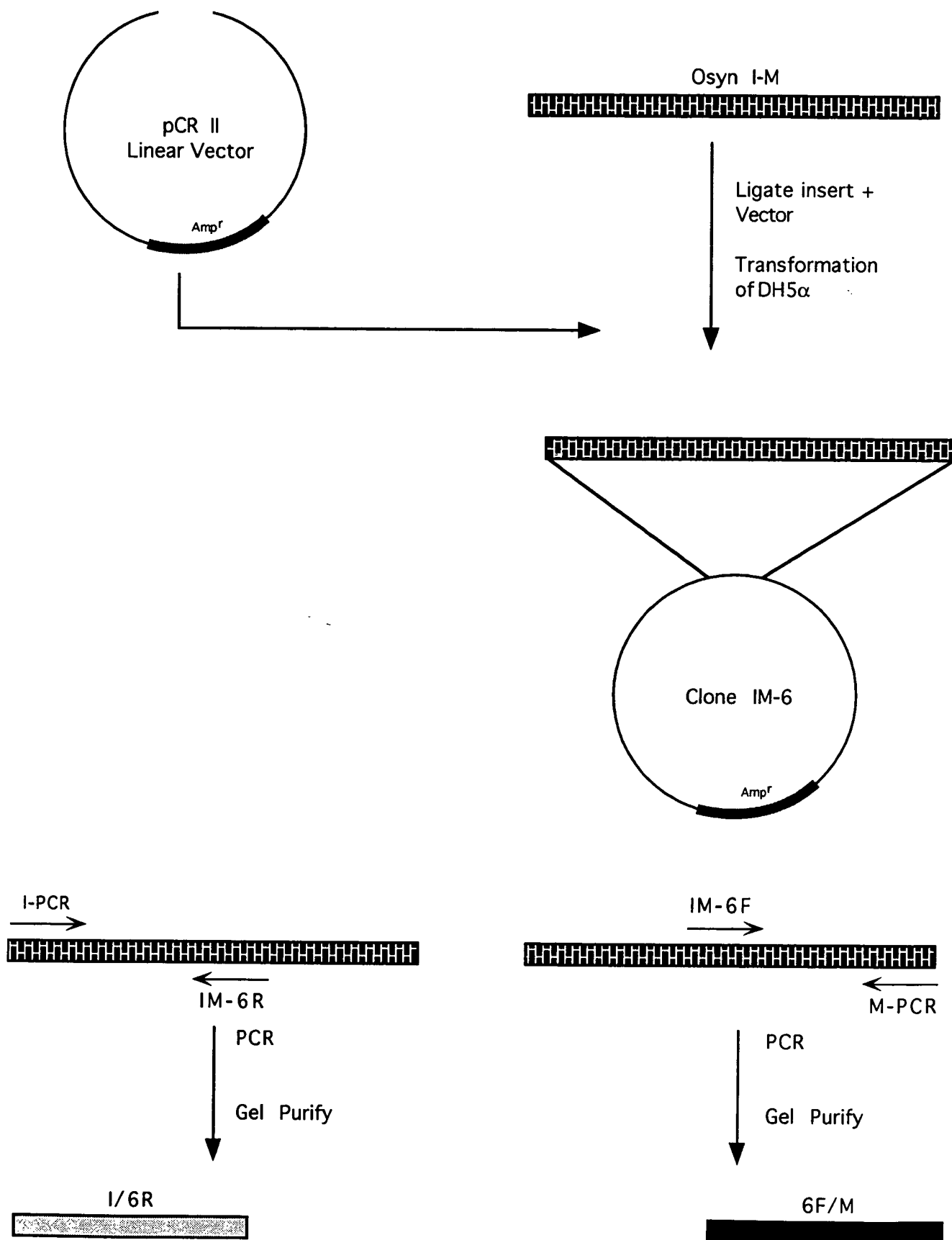


Fig 4E



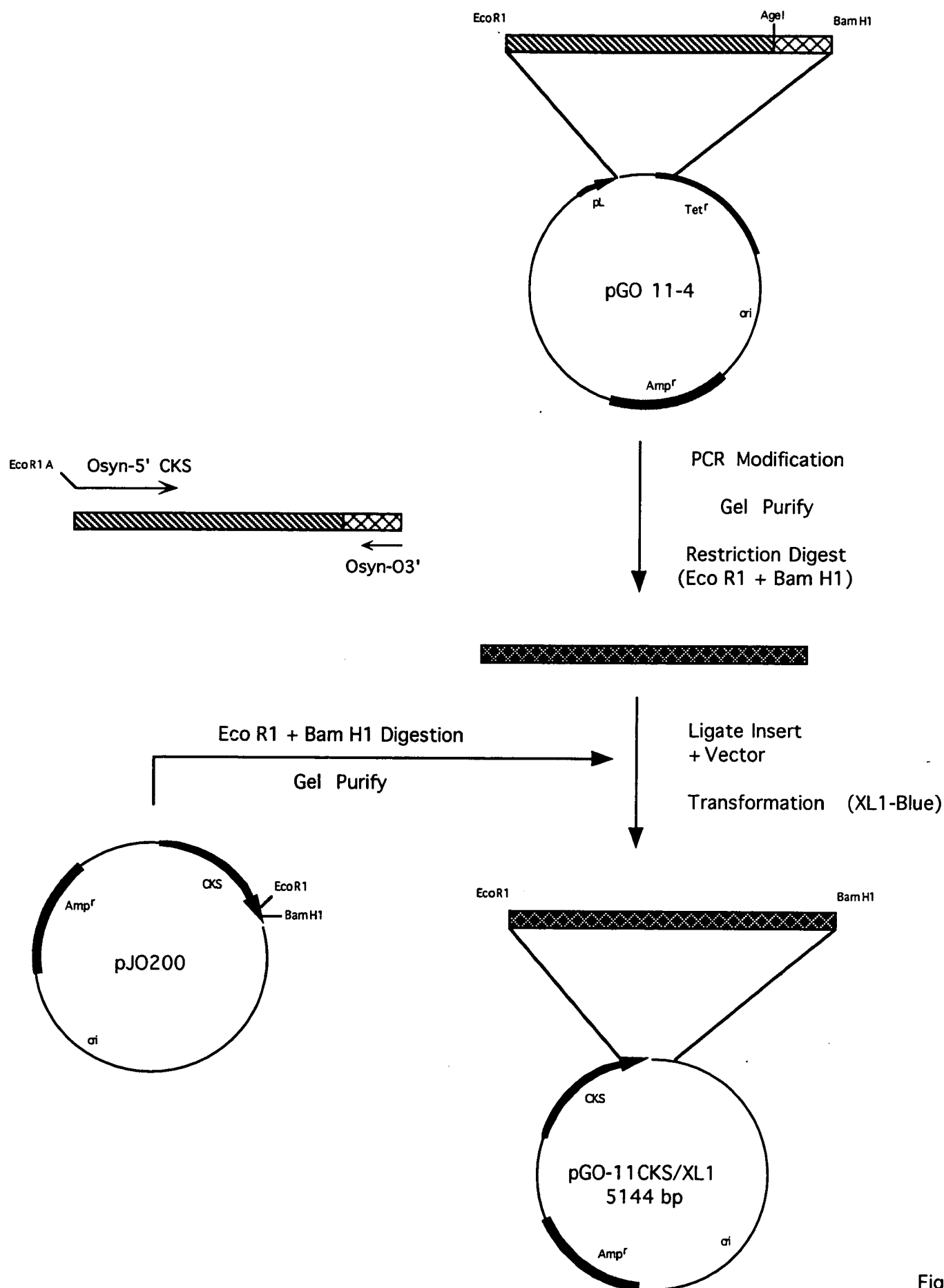


Fig 4G

# Figure 5

└─> gp120  
MIGGDMKDIW RNELFKYKVV RVKPFVAPT PIARPVIGTG THREKRAVGL 50  
└─> gp41  
GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQL 100  
LRLSVWGIRQ LRARLLALET LIQNQQLLNL WGCKGRLICY TSVKWNETWR 150  
NTTNINQIWG NLTWQEQDQQ IDNVSSTIYE EIQKAQVQQE QNEKKLLELD 200  
EWASLWNWLD ITKWL 215

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# Figure 6

→ CKS

MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIIVATD 50  
HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100  
EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG 150  
YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIIYGYRAG FIRRYVNWQP 200  
SPLEHIEMLE QLRVLWYGEK IHVAVAQEVV GTGVDTPEL DPSTNSIGGD 250  
MKDIWRNELF KYKVVRVKPF SVAPTPIARP VIGTGTHREK RAVGLGMLFL 300  
GVLSAAGSTM GAAATALTVQ THSVIKGIVQ QQDNLLRAIQ AQQELLRLSV 350  
WGIRQLRARL LALETLIQNQ QLLNLWGCKG RLICYTSVKW NETWRNTTNI 400  
NQIWGNLTWQ EWDQQIDNVS STIYEEIQKA QVQQEQNEKK LLELDEWASL 450  
WNWLDITKWL 460

→ gp120  
→ gp41

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# Figure 7

→ gp120 → gp41  
MIGGDMKDIW RNELFKYKVV RVKPF SVAPT PIARPVIGTG THREKRAVGL 50  
GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQEL 100  
LRLSVWGIRQ LRARLLALET LIQNQQLLNL WGCKGRLICY TSVKWNETWR 150  
NTTNINQIWG NLTWQEW DQQ IDNVSSTIYE EIQKAQVQQE QNEKKLLELD 200  
EWASLWNWLD ITKWLRNIRQ GYQPLSLQIP TRQQSEAETP GRTGE 245

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# Figure 8

➤ CKS

MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIIVATD 50  
HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100  
EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG 150  
YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIIYGYRAG FIRRYVNWQP 200  
SPLEHIEMLE QLRVLWYGEK IHVAVAQEVV GTGVDTPEDL DPSTNSIGGD 250  
MKDIWRNELF KYKVVRVKPF SVAPTPIARP VIGTGTHREK RAVGLGMLFL 300  
GVLSAAGSTM GAAATALTVQ THSVIKGIVQ QQDNLLRAIQ AQQELLRLSV 350  
WGIRQLRARL LALETLIQNQ QLLNLWGCKG RLICYTSVKW NETWRNTTNI 400  
NQIWGNLTWQ EWDQQIDNVS STIYEEIQKA QVQQEQNEKK LLELDEWASL 450  
WNWLDITKWL RNIRQGYQPL SLQIPTRQQS EAETPGRTGE 490

➤ gp120

➤ gp41

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# Figure 9

→ gp120  
MIGGDMKDIW RNELFKYKVV RVKPFVAPT PIARPVIGTG THREKRAVGL 50  
→ gp41

GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQL 100

LRLSVWGIRQ LRARLLALET LIQNQQLNL WGCKGRLICY TSVKWNETWR 150

NTTNINQIWG NLTWQEWQQ IDNVSSSTIYE EIQKAQVQQE QNEKKLLELD 200

EWASLWNWLD ITKWL|RNIRQ GYQPLSLQIP TRQQSEAETP GRTGEGGGDE 250

GRPRLIPSPQ GFLPLLYTDL RTIILWSYHL LSNLISGTQT VISHLRLGLW 300

ILGQKIIDAC RICA AVIHYW LQELQKSATS LIDTFAVAVA NWTDDIILGI 350

QRLGRGILNI PRRVRQGFER SLL 373

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# Figure 10

➤ CKS

MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIIVATD 50

HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100

EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG 150

YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIIYGYRAG FIRRYVNWQP 200

SPLHEIEMLE QLRVLWYGEK IHVAVAQEVV GTGVDTPEDL DPSTNSIGGD 250

➤ gp120

MKDIWRNELF KYKVVRVKPF SVAPTPIARP VIGTGTHREK RAVGLGMLFL 300

➤ gp41

GVLSAAGSTM GAAATALTVQ THSVIKGIVQ QQDNLLRAIQ AQQELLRLSV 350

WGIRQLRARL LALETLIQNQ QLLNLWGCKG RLICYTSVKW NETWRNTTNI 400

NQIWGNLTWQ EWDQQIDNVS STIYEEIQKA QVQQEQNEKK LLELDEWASL 450

WNWLDITKWL RNIRQGYQPL SLQIPTRQQS EAETPGRTGE GGGDEGRPRL 500

IPSPQGFLPL LYTDLRTIIL WSYHLLSNLI SGTQTVISHL RLGLWILGQK 550

IIDACRICAA VIHYYWLQELQ KSATSLIDTF AVAVANWTDD IILGIQRLGR 600

GILNIPRRVR QGFERSLL 618

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# Figure 11

→ CKS  
MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIIVATD 50  
HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100  
EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG 150  
YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIIYGYRAG FIRRYVNWQP 200  
SPLEHIEMLE QLRVLWYGEK IHVAVAQEVV GTGVDTPEDL DPSTNSMEGE 250  
LTCNSTVTSI IANIDSDGNQ TNITFSAEVA ELYRLELG DY KLIEVTPIGF 300  
APTKEKRYSS APVRNKRGVF VLGFLGFLAT AGSAMGAASL TLSAQSR TLL 350  
AGIVQQQQQL LDVVKRQQEM LRLTVWG TKN LQARVTAIEK YLKDQAQLNS 400  
WGCAFRQVCH TTPVWVND SL TPDWNNMTWQ EWEKRVHYLE ANISQSLEQA 450  
QIQQEKNMYE LQKLNS 466

→ gp120  
→ gp36

08911304-01597

## Figure 12

➤ CKS  
MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIIVATD 50  
HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100  
EPMIPATIIR QVADNLAQRQ VGMATLAVPI HNAEEAFNPN AVKVVLDAEG 150  
YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIIYGYRAG FIRRYVNWQP 200  
SPLEHIEMLE QLRVLWYGEK IHVAVAQEVV GTGVDTPEDP STALMKIPGD 250  
➤ gp120  
PGGGDMRDNW RSELYKYKVV KIEPLGVAPT KAKRRVVQRE ➤ gp41 KRAVGIGALF 300  
LGFLGAAGST MGAASMTLTV QARQLLSGIV QQQNNLLRAI EAQQHLLQLT 350  
VWGIKQLQAR ILAVERYLKD QQLLGIWGCS GKLICTTAVP WNASWSNKSL 400  
EQIWNNMTWM EWDREINNYT SLIHSLIEES QNQQEKNEQE LLELDKWVNR 450  
VRQGYSPLSF QTHLPIPRGP DRPEGIEEEG GERDRDRSIR LVNGSLALIW 500  
DDLRSCLCLS YHRLRDLILLI VTRIVELLGR RGWEALKYWW NLLQYWSQEL 550  
KNSAVSLLNA TAIAVAEGTD RVIEVVQGAY RAIRHIPRRI RQGLERILL 599

06911924-061597

# Figure 13

→ CKS  
MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIIVATD 50  
HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100  
EPMIPATIIR QVADNLAQRQ VGMATLAVPI HNAEEAFNPN AVKVVLDAEG 150  
YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIIYGYRAG FIRRYVNWQP 200  
SPLEHIEMLE QLRVLWYGEK IHVAVAQEVPT GTGVDTPEDP STALMKIPGD 250  
→ gp120 (HXB2R) → gp41 (HXB2R)  
PGGGDMRDNW RSELYKYKV KIEPLGVAPT KAKRRVVQRE KRAVGIGALF 300  
LGFLGAAGST MGAASMTLTV QARQLLSGIV QQQNNLLRAI EAQQHLLQLT 350  
VWGIKQLQAR ILAVERYLKD QQLLGIWGCS GKLICTTAVP WNASWSNKSL 400  
EQIWNMTWM EWDREINNYT SLIHSLIEES QNQQEKNEQE LLELDKWVNR 450  
VRQGYSPLSF QTHLPIPRGP DRPEGIEEEG GERDRDRSIR LVIGGDMKDI 500  
→ gp120 (HAM112)  
WRNELFKYKV VRVKPFSVAP TPIARPVIGT GTHREKRAVG LGMLFLGVLS 550  
→ gp41 (HAM112)  
AAGSTMGAAA TALTVQTHSV IKGIVQQQDN LLRAIQAQQE LLRLSVWGIR 600  
QLRARLLALE TLIQNQQLLN LWGCKGRLIC YTSVKWNETW RNTTNINQIW 650  
GNLTWQEQWDQ QIDNVSSTIY EEIQKAQVQQ EQNEKKLLEL DEWASLWNWL 700  
DITKWLRNIR QGYQPLSLQI PTRQQSEAET PGRTGE 736

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# Figure 14

➤ CKS  
 MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIIVATD 50  
 HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100  
 EPMIPATIIR QVADNLAQRQ VGMATLAVPI HNAEEAFNPN AVKVVLDAEG 150  
 YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIIYGYRAG FIRRYVNWQP 200  
 SPLEHIEMLE QLRVLWYGEK IHVAVAQEVPT GTGVDTPEDP STALMKIPGD 250  
 ➤ gp120 (HXB2R)      ➤ gp41 (HXB2R)  
 PGGGDMRDNW RSELYKYKVV KIEPLGVAPT KAKRRVVQRE KRAVGIGALF 300  
 LGFLGAAGST MGAASMTLTV QARQLLSGIV QQQNNLLRAI EAQQHLLQLT 350  
 VWGIKQLQAR ILAVERYLKD QQLLGIWGCS GKLICTTAVP WNASWSNKSL 400  
 EQIWNMTWM EWDREINNYT SLIHSLIEES QNQQEKNEQE LLELDKWVNR 450  
 VRQGYSPLSF QTHLPIPRGP DRPEGIEEEG GERDRDRSIR LVIGGDMKDI 500  
 ➤ gp120 (HAM112)  
 WRNELFKYKV VRVKPFVAP TPIARPVIGT GTHREKRAVG LGMLFLGVLS 550  
 ➤ gp41 (HAM112)  
 AAGSTMGAAA TALTVQTHSV IKGIVQQQDN LLRAIQAQQE LLRLSVWGIR 600  
 QLRARLLALE TLIQNQQLLN LWGCKGRLIC YTSVKWNETW RNTTNINQIW 650  
 GNLTWQWDQ QIDNVSSIIY EEIQKAQVQQ EQNEKKLLEL DEWASLWNWL 700  
 DITKWL 706

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# Figure 15

→ gp120 (HAM112) → gp41 (HAM112)  
MIGGDMKDIW RNELFKYKVV RVKPFVAPT PIARPVIGTG THREKRAVGL 50  
GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQEL 100  
LRLSVWGIRQ LRARLLALET LIQNQQLLNL WGCKGR LICY TSVKWNETWR 150  
NTTNINQIWG NLTWQEW DQQ IDNV SSTIYE EIQKAQVQQE QNEKKLLELD 200  
EWASLWNWLD ITKWLRNIRQ GYQPLSLQIP TRQQSEAETP GRTGEGP GGG 250  
DMRDNWRSEL YKYKVV KIEP LGVAPT KAKR RVVQREKRAV GIGALFLGFL 300  
GAAGSTMGAA SMTLTVQARQ LLSGIVQQQN NLLRAIEAQQ HLLQLTVWGI 350  
KQLQARILAV ERYLKDQQLL GIWGCSGKLI CTTAVPWNAS WSNKSLEQIW 400  
NNMTWMEWDR EINNYTSLIH SLIEESQNQQ EKNEQELLEL DKWVNRVRQG 450  
YSPLSFQTHL PIPRGPDRPE GIEEEGGERD RDRSIRLV 488

269130-469130



# Figure 16

→ CKS  
MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIVATD 50  
HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100  
EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG 150  
YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIIYGYRAG FIRRYVNWQP 200  
SPLEHIEMLE QLRVLWYGEK IHVAVAQEV GTGVDTPEDL DPSTNSIGGD 250  
MKDIWRNELF KYKVVRVKPF SVAPTPIARP VIGTGTHREK RAVGLGMLFL 300  
GVLSAAGSTM GAAATALTVQ THSVIKGIVQ QQDNLLRAIQ AQQELLRLSV 350  
WGIRQLRARL LAETLIQNQ QLLNLWGCKG RLICYTSVKW NETWRNTTNI 400  
NQIWGNLTWQ EWDQQIDNVS STIYEEIQKA QVQQEQNEKK LLELDEWASL 450  
WNWLDITKWL RNIRQGYQPL SLQIPTRQQS EAETPGRTGE GGGSRLLALE 500  
TLIQNQQLLN LWGCKGRLIC YTSVKW 526

→ gp120 (HAM112)  
→ gp41 (HAM112)  
→ linker → IDR (HAM112)

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# Figure 17

→ gp120 (HAM112) → gp41 (HAM112)

MIGGDMKDIW RNELFKYKVV RVKPFVAPT PIARPVIGTG THREKRAVGL 50

GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQEL 100

LRLSVWGIRQ LRARLLALET LIQNQQLLNL WGCKGR LICY TSVKWN ETWR 150

NTTNINQIWG NLTWQEW DQQ IDNVSSTIYE EIQKAQVQQE QNEKKLLELD 200

EWASLWNWLD ITKWLRNIRQ GYQPLSLQIP TRQQSEAETP GRTGEGGGSR 250

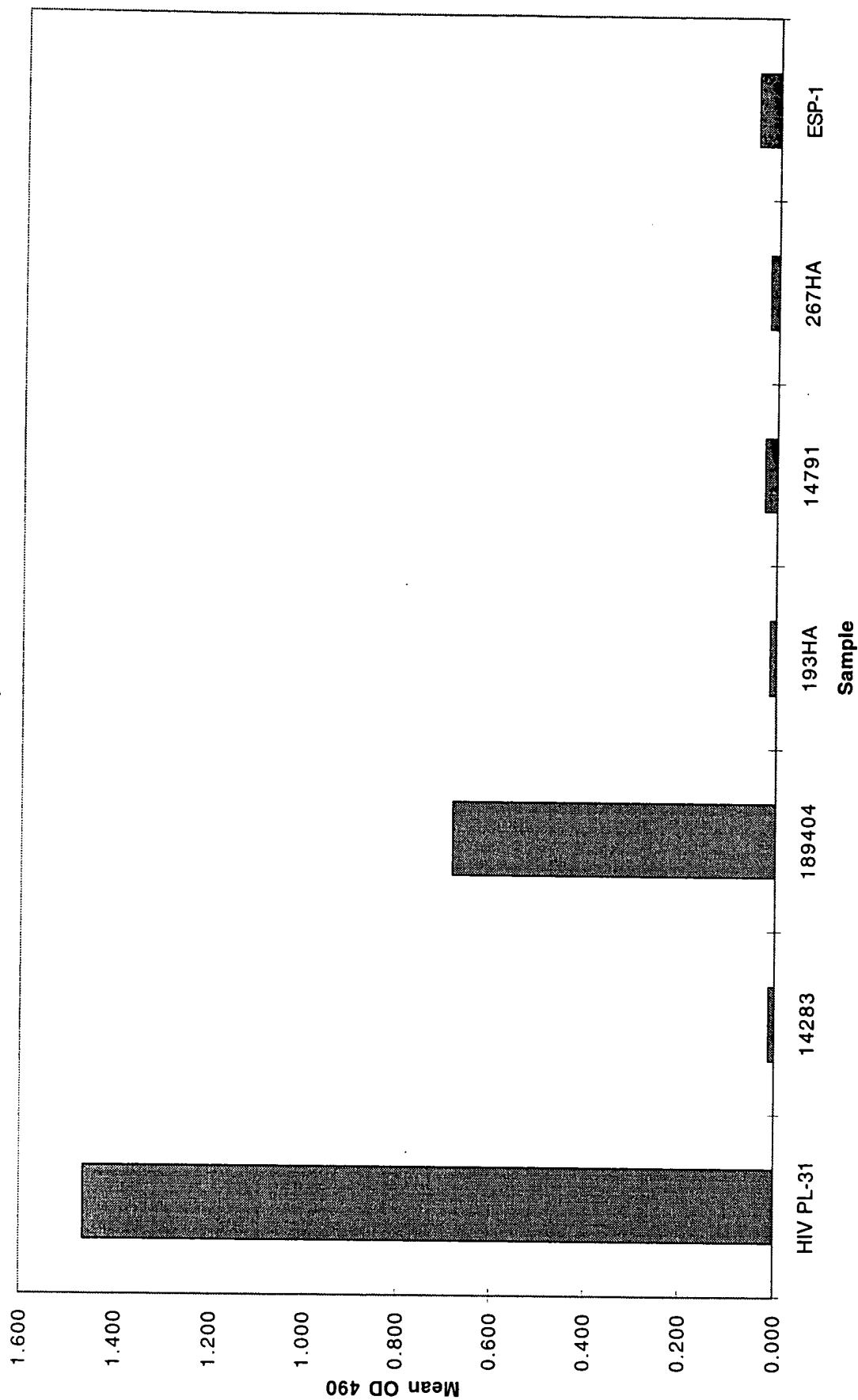
LLALET LIQN QQLLNLWGCK GR LICYTSVK W 281

linker IDR (HAM112)

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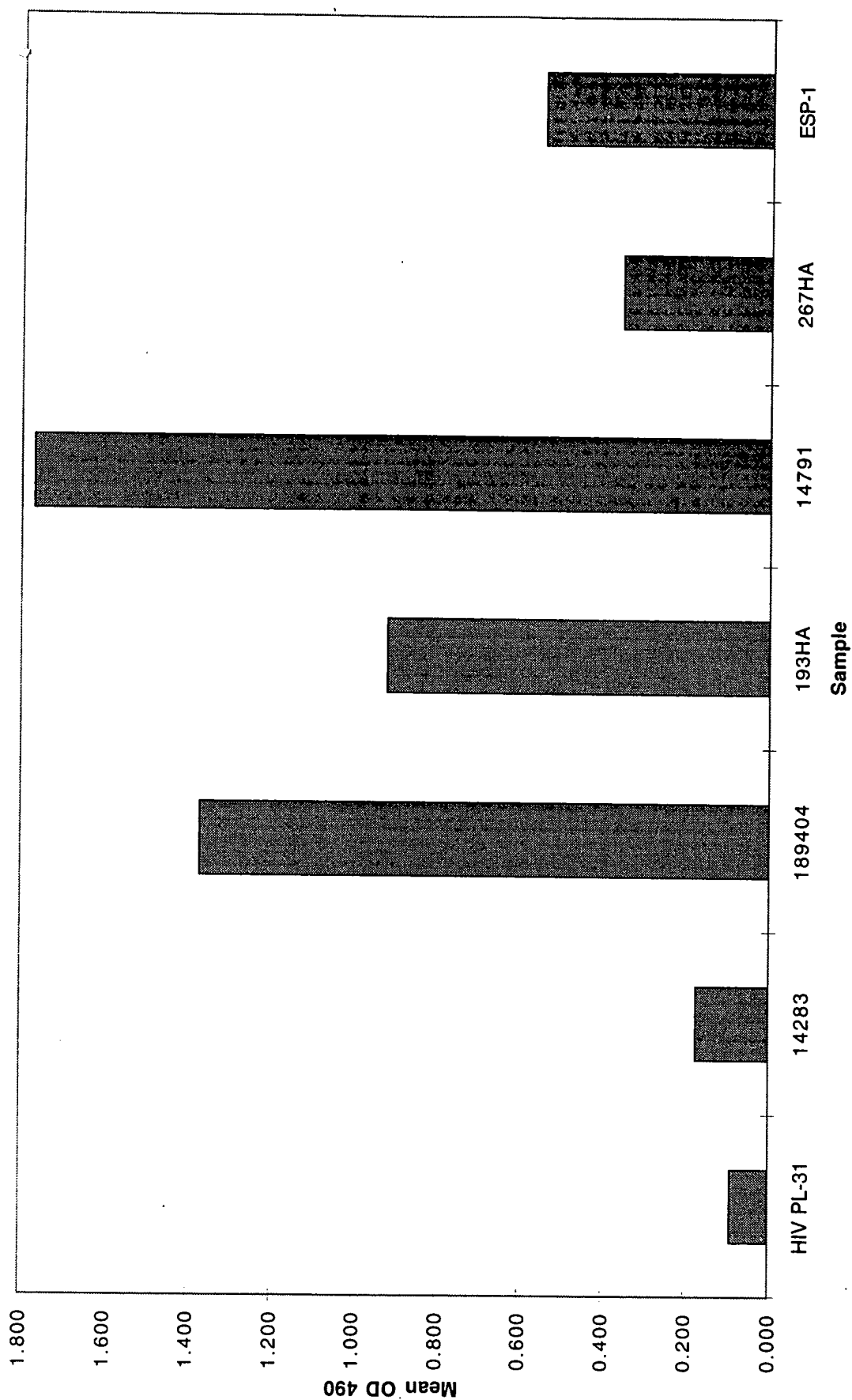
**Figure 18**

**pTB319 Bead  
1:100,000**



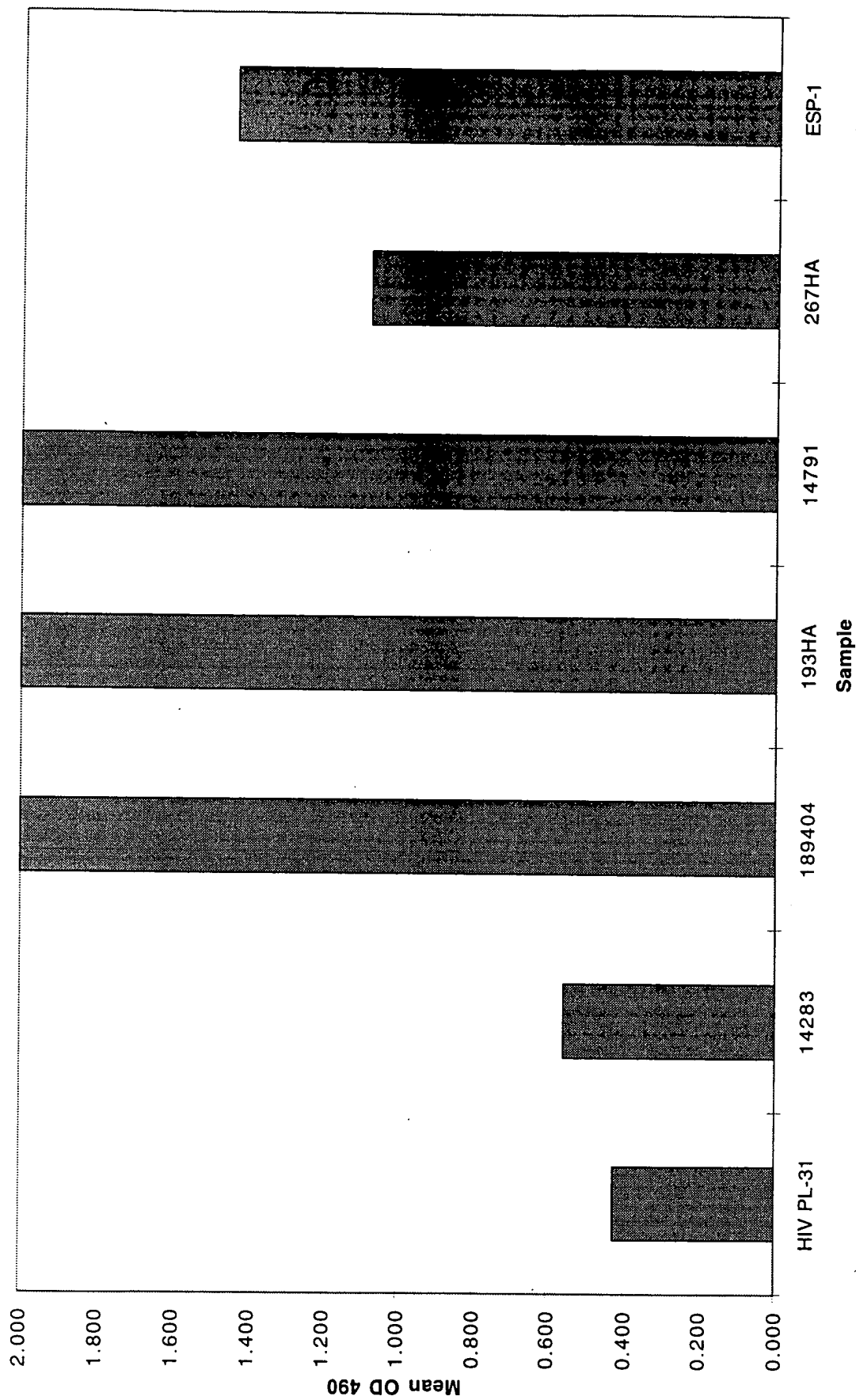
**Figure 19**

**pGO-9/CKS Bead  
1:10,000**



**Figure 20**

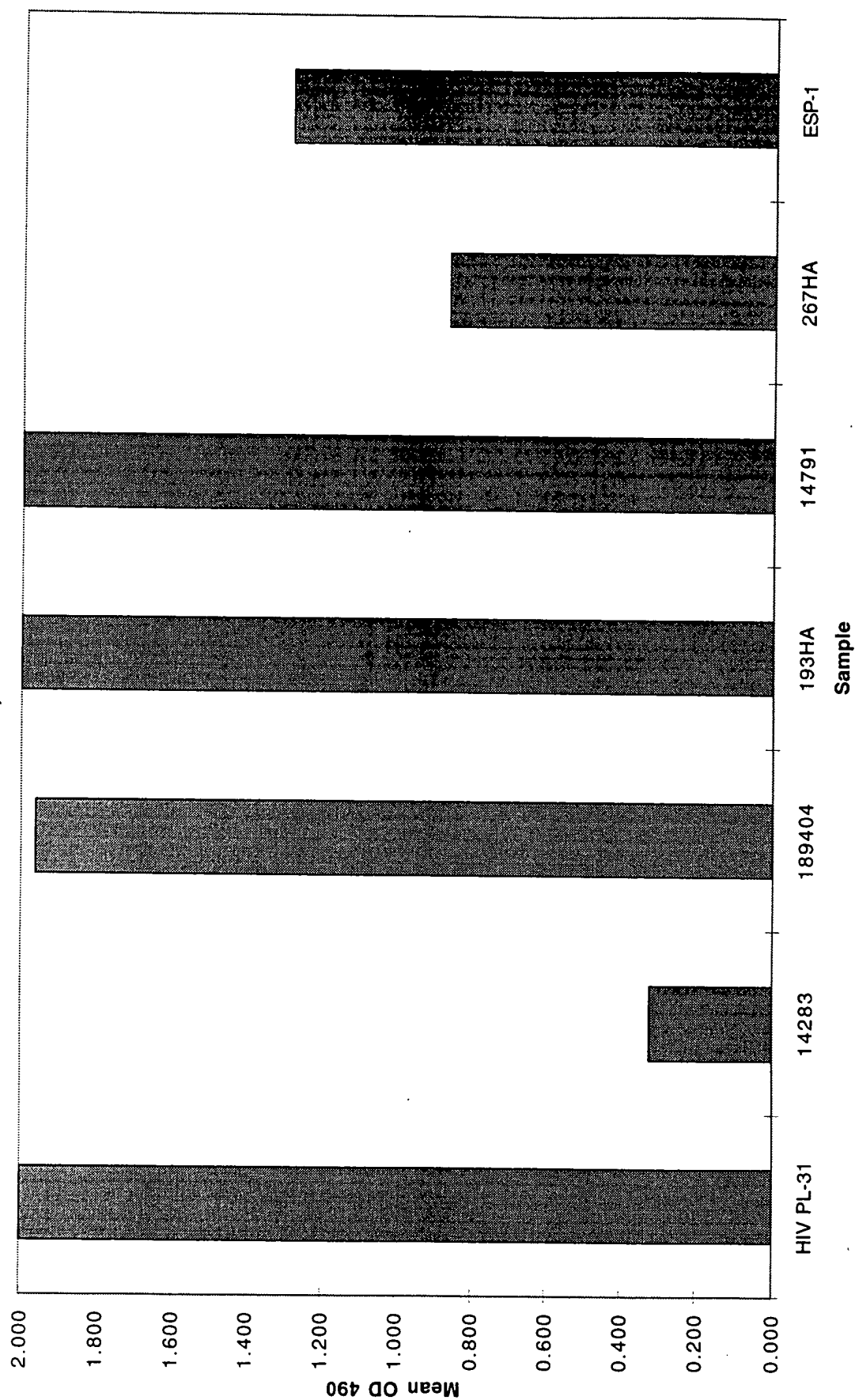
pGO-11/PL Bead  
1:10,000



**Figure 21**

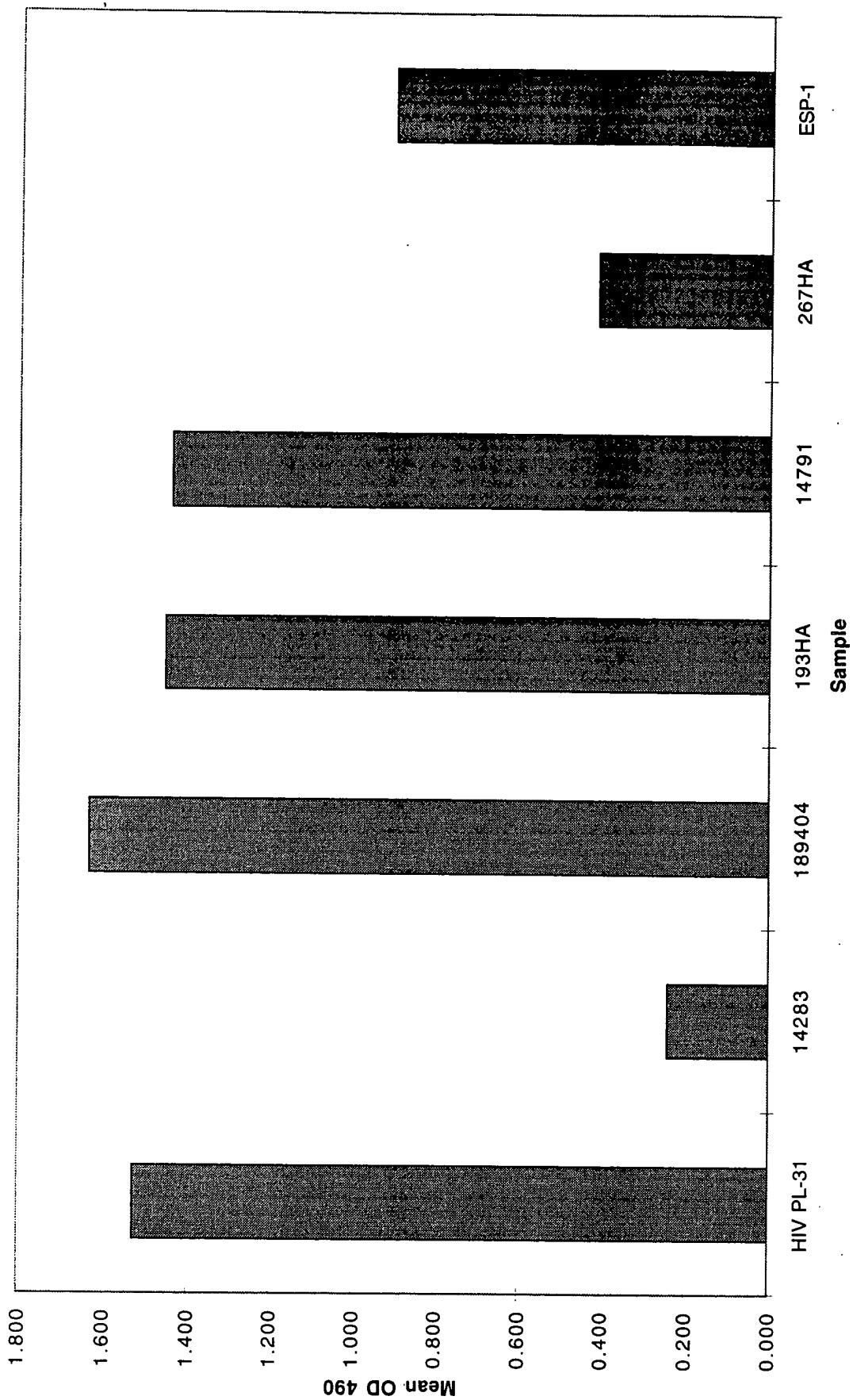
**pGO-12/CKS Bead**

**1:10,000**



**Figure 22**

**pGO-14/PL Bead  
1:10,000**



**Figure 23**

**pGO-15/CKS  
1:10,000**

